Responses to Public Comments and Peer Reviews

Phase III: Diuron Criteria Derivation Report

using the

Phase II: Methodology for Derivation of Pesticide Water Quality Criteria for the Protection of Aquatic Life in the Sacramento and San Joaquin River Basins

Tessa L. Fojut, Ph.D.
and
Ronald S. Tjeerdema, Ph.D.

Department of Environmental Toxicology
University of California, Davis

March 2010

THIS PAGE INTENTIONALLY BLANK

Table of Contents

Terms, Abbreviations, Acronyms, and Initialisms Used in this Report

Term	Definition
ACR	Acute to Chronic Ratio- used to estimate concentration that will protect against chronic toxicity
CDFG	California Department of Fish and Game
CVRWQCB	Central Valley Regional Water Quality Control Board
DPR	California Department of Pesticide Regulation
EC*x*	The chemical concentration that has an effect on *x*% of the test population.
Koc	Organic Carbon Partition Coefficient
LC50	The chemical concentration that is lethal to 50 % of the test population.
LOEC	Lowest Observed Effect Level- lowest concentration tested that has some effect on the test population
MATC	Maximum Allowable Toxicant Concentration -geometric mean of LOEC and NOEC
NOEC	No Observed Effect Level- highest concentration tested that has no effect on the test population
SSD	Species Sensitivity Distribution- Statistical probability distribution of toxicity data
UC Davis	University of California, Davis
US EPA	U.S. Environmental Protection Agency
Water Quality Objective (WQO)	The limits of water quality constituents or characteristics that are established for the reasonable protection of beneficial uses of water or the prevention of nuisance within a specific area.

1.0 Introduction

This document presents the responses to public comments and peer reviews received on a technical report prepared by the University of California at Davis, Environmental Toxicology Department, under contract (#05-100-150-0) to the Regional Water Quality Control Board, Central Valley Region (Regional Board). This report represents one of six the end product reports of the third phase of a three-phase project to evaluate, develop and apply a method to derive pesticide water quality criteria for the protection of aquatic life.

The first phase of the project was to review and evaluate existing water quality criteria derivation methodologies to determine if there was an existing available method that met the Regional Board's stated project goals. The review indicated that there is no single method that meets all of the Regional Boards requirements. Therefore, the second phase of the project was to develop a new method that could meet the project requirements. The Phase II report details this new methodology and its application to chlorpyrifos. The third phase of the project was to apply the criteria derivation method to six additional pesticides, of which diuron is one.

The diuron criteria report was submitted to peer review, conducted by experts from academia and sister agencies, including the Department of Fish and Game and the Department of Pesticide Regulation.

These technical reports may be considered by the Regional Board during the development of the Central Valley Pesticide Basin Plan Amendment or other Board actions. However, the reports do not represent Board Policy and are not regulations. The reports are intended to generate numeric water quality criteria for the protection of aquatic life. However, these should not be construed as water quality objectives. Criteria and guidelines do not have the force and effect of regulation, nor are they themselves water quality objectives.

2.0 Response to Comment to Public Comments

2.1. Comment Letter 1 – Lenwood Hall, University of Maryland

COMMENT 1-1: In my view, the step by step process for reviewing the toxicity data is cumbersome and somewhat flawed. In the current format, a total of 4 forms need to be completed if the relevance score in Table 3.6 is ≥70. It would be more logical to first establish criteria that **must** be acceptable before conducting any other evaluation of documents containing the toxicity data. These "Kill Switch Criteria" that must be met for an acceptable study are as follows: (1) Is the control endpoint (survival or growth) acceptable?; (2) Is the document under review the (primary) original source of the data?; (3) Were adverse effects evaluated using exposures of a single pesticide?; (4) Was the duration of exposure reported?; (5) Were the effects reported for relevant endpoints (e.g., survival, growth, or reproduction)?; (6) Was more than one dose/concentration used in the toxicity test?; (7) Was the test species reported?; (8) Was the chemical form (% active ingredient) of the test material reported?; and (9) Was a dose response relationship evident? For example, in the current data review process a study with unacceptable control survival receives a 7.5 point reduction (see Table 3.6 in TenBrook et al. 2009) and can still be rated acceptable for criteria development.

Response To Comment (RTC) 1-1: The data evaluation process of the methodology has been thoroughly reviewed by both peer review and public comment processes, but may be revised in the future.

COMMENT 1-2: In Table 3.11 from TenBrook et al. 2009 studies receive scores for both relevance and reliability as follows: N = not relevant/not reliable; L = less relevant/reliable; and R = relevant, reliable. Only scores rated relevant and reliable (RR) are used for criteria derivation as described in TenBrook et al. 2009. However, when the preliminary acute criterion of 168 ug/L was derived on the bottom of page 6 additional analysis was then conducted comparing this value of 168 ug/L with a *Gammarus lacustris* acute value of 160 ug/L despite the fact that the *G. lacustris* study was rated LL (less relevant, less reliable). In other words, a study that was not rated RR and judged unacceptable for criteria development could be used to drive the final criterion. This is illogical and would negate the entire data quality review process.

RTC 1-2: Clarification on the use of supplemental data (studies rated RL, LR, or LL) in criteria adjustment has been added to the Sensitive Species section of the

report. Section 3-6.0 of the methodology, titled “Check criteria against ecotoxicity data,” describes how the criteria are evaluated to ensure they are protective to: 1) particularly sensitive species, 2) ecosystems, and 3) threatened and endangered species (TenBrook *et al.* 2009). Supplemental data are used to evaluate the criteria, particularly for sensitive species, as described in section 3-6.1 of the methodology, because there may be particularly sensitive species in the supplemental data set that are not well-represented in the acceptable data set (studies rated RR), from which the criteria are calculated. It is stated in this section (3-6.1): if the calculated criterion is higher than measured toxicity values reported for particularly sensitive species, then the criterion may require downward adjustment (TenBrook *et al.* 2009). It is noted in the Acute Criterion Calculation section of the report that the assessment factor procedure should be used with caution for diuron because the assessment factors were developed with data from neurotoxic insecticides, not herbicides, which is why the criterion was evaluated to check if it would be protective based on the entire data set. In the final report the acute criterion is rounded to two significant digits, and is no longer adjusted downward, because the *Gammarus lacustris* data is based on nominal concentrations, and the method specifies that criterion adjustment should be based on studies with measured concentrations.

> **COMMENT 1-3:** The use of a safety factor of 36 for a herbicide, such as diuron, because only 2 acceptable data points were available is questionable (see page 6). This safety factor of 36, as discussed in TenBrook et al. 2009, was based on data from organic insecticides which have different modes of action than herbicides, such as diuron, so there is no scientific rationale for using this safety factor. Perhaps discussions with registrants about filling these data gaps should be pursued in order to make this a more data driven process and avoid the use of unreliable safety factors.

RTC 1-3: It is stated in the Acute Criterion section of the report that the Assessment Factor approach should be used with caution for an herbicide. However, diuron is a chlorinated compound that does exhibit toxicity to animals, although the mechanism is not clear. The AFs given in the methodology (Table 3.13, TenBrook *et al.* 2009a) are the most specific AFs available for organic pesticides, compared to those used in the Great Lakes Initiative (USEPA 2003), which derived AFs from a much broader array of chemicals, such as metals and industrial chemicals. The AFs in the UC-Davis methodology were derived using animal toxicity data, and thus are not used to derive the chronic criterion for an herbicide because they were not calculated with any plant toxicity data.

We requested additional plant toxicity data from the manufacturer of diuron, and they submitted the studies to the EPA. We have requested the studies from EPA, but have only received one of the studies at the time of publication of this report, which was not enough to use a SSD procedure for the chronic criterion

calculation. When these studies are available, the data should be evaluated and incorporated into a revised criteria report.

> **COMMENT 1-4:** Since plant data are only used for a chronic criterion with herbicides there is uncertainty when using NOEC, LOEC, and MATC values because these values will be determined by the range of test concentrations (dilution series) and the sample size used in the toxicity test. There are numerous papers in the peer reviewed literature discussing the uncertainty associated with NOEC, LOEC and MATC values in the regulatory process because these values have no statistical confidence (Newman, 2010; among others). For example, a suboptimal design with low statistical power and high error variances may produce higher NOEC and LOEC values in contrast to a superior design that may produce lower NOEC and LOEC values. This is a critical issue because the NOEC value of 1.3 ug/L from a plant toxicity test with *Pseudokirchneriella subcapitata* is used as the final chronic criterion. It is also noteworthy that the UC Davis methodology is in direct conflict with EPA (USEPA, 2003) on this point as EPA uses the same green algae study used in this report as the only acceptable plant data for diuron. However, EPA uses the EC50 value of 2.4 ug/L. I would suggest using either an EC50 or an EC20 value (if it can be calculated) for developing the final chronic criterion and not the NOEC.

RTC 1-4: EC_x values cannot be used to calculate the chronic criterion for diuron because studies are not available that show what level of *x* is appropriate to represent a no-effect level (section 3-2.1.1.2, TenBrook *et al.* 2009). Toxicity values from hypothesis tests are evaluated to ensure that they are reasonable estimates of no-effect levels (section 2-2.1.2 and Tables 3.7 and 3.8, TenBrook *et al.* 2009). The EPA used the EC_{50} value to calculate a benchmark value, which is not the same as a water quality criterion. The US EPA (1985) criteria derivation methodology also recommends the use of the lowest plant toxicity value to derive the chronic criterion for an herbicide.

> **Comment 1-5:** If the control response is adequate and the test is considered valid it seems unreasonable to deduct points in the data evaluation process and require acceptable: (1) tolerance ranges for various water quality parameters (e.g., hardness, alkalinity, conductivity, pH); (2) dilution water information; and (3) information on no prior contaminant exposure (rarely mentioned in a document). In many cases the tolerance ranges for water quality parameters such as hardness, alkalinity, conductivity and pH are simply unknown for a test species. I am concerned that valid toxicity studies could be graded as unacceptable if the current data review process includes the parameters described above.

RTC 1-5: The data evaluation process of the methodology has been thoroughly reviewed by both peer review and public comment processes, but may be revised in the future.

COMMENT 1-6: There seems to be at least some issues with the transparency of this water quality criteria development process. For example in TenBrook et al. 2009 the following points are given in Table 3.7 for reporting these water quality parameters: hardness (2 points), alkalinity (2 points), dissolved oxygen (4 points), temperature (4 points), conductivity (2 points), and pH (3 points). However, in the current diuron water quality criteria document it is stated on the top of page 5 that dissolved oxygen, hardness, alkalinity, and conductivity were not considered in the reliability assessment and full points were given for these parameters because these parameters are not relevant for plant studies. If these parameters are not considered important for plant study scoring then the original TenBrook et al. 2009 document needs to be changed to address this point.

RTC 1-6: We have re-rated the studies in which some water quality parameters were omitted from scoring, which is reflected in changes to the data tables. Data summary sheets are available for every study in the Appendix and complete scoring for each study is listed at the bottom of each summary sheet.

COMMENT 1-7: Page 4, line 13 – The authors state that 84 studies were identified with diuron toxicity data. Does this mean that there were toxicity data for 84 different species?

RTC 1-7: We identified 84 studies with diuron toxicity data, some studies contained data for multiple species, or multiple tests. Each toxicity value reported in a study is listed separately in the data tables (Tables 4-9).

COMMENT 1-8: Page 16, parag 4, line 2 – It is stated that the 4-h averaging period should be protective based on available data. This should be 4-d not 4- h.

RTC 1-8: This has been corrected in the report.

COMMENT 1-9: Appendix - The data summary forms in the Appendix summarize the relevance and reliability scoring and the notes section briefly mentions where points were lost for various parameters. However, it would be much easier and more transparent for the interested reader if the authors were to include the actual scoring for all the forms in Table 3.6, Table 3.7 and Table 3.8 within the appendix for each species.

RTC 1-9: The list of scoring at the end of each data summary form is an exact list of which points were taken off according to Tables 3.7 and 3.8 in the methodology. We document them in a concise list so that the scoring of each study is completely transparent.

2.2. Comment Letter 2 – Nasser Dean, Western Plant Health Association

COMMENT 2-1: WPHA restates for the written record our previous concerns about the CVRWQCB embarking on an expeditious and narrowly focused policy towards developing an excessively conservative WQC Method for 7 active ingredients to then be applied to listed "waterbodies" just within the Central Valley. This initiative would be subject to rigorous monitoring and compliance activities through your agency's regulatory enforcement against growers/agricultural dischargers. We would respectfully suggest, once again, that the CVRWQCB staff would be judicious in redirecting their attention to the ongoing harmonization effort between the Clean Water Act (CWA) and the Federal Insecticide, Fungicide and Rodenticide Act **(**FIFRA**)** by the United States Environmental Protection Agency (US EPA) Office of Water (OW) and Office of Pesticide Programs (OPP). As you may be aware, beginning in 2010, the OW/OPP harmonization effort will have a series of public workshops throughout the United States that will attempt to solicit input from a variety of technical stakeholders on how best to address the lingering issue of limited aquatic toxicity datasets from pesticides. The unified outcome may prove both fruitful and scientifically justifiable to permit its use by each of the States.

RTC 2-1: The comparison of criteria outcomes of the UCD methodology, US EPA, and CDFG reports for diazinon and chlorpyrifos indicate that the UCD methodology derives criteria very similar to those of other agencies, which are regarded as reasonable water quality criteria, and not excessively conservative. The rationale for this project is described in the Phase I report (TenBrook & Tjeerdema 2006). We are aware of the OW/OPP harmonization effort and look forward to the results, which will likely improve water quality criteria derivation efforts by all agencies.

COMMENT 2-2: As the authors for this WQC method (Fojut et al.) had noted, some of the data quality criteria are not appropriate to plant studies and applying the method to aquatic plant data revealed challenges both to the review process and to the selection of endpoints.

RTC 2-2: The data evaluation process of the methodology has been thoroughly reviewed by both peer review and public comment processes, but may be revised in the future.

COMMENT 2-3: The extensive data review for diuron emphasizes that studies conducted by registrants and submitted to meet pesticide registration data requirements of the US EPA and other regulatory agencies are appropriate for establishing environmental quality criteria. The two studies selected to establish the acute and chronic criteria (Baer,

1991 and Blasberg, 1991) were conducted by the registrant, submitted to the US EPA, and reviewed by the US EPA. US EPA deemed the studies acceptable for meeting regulatory requirements. While research studies published in the peer-reviewed literature may be acceptable for consideration in setting environmental criteria, it is critical that data meet the standards required for consistency in regulatory decisions, whether at the national, state or regional level. WPHA encourages Dr. Fojut et al. to continue working with registrants to identify additional data that meet the goals of the criterion setting process.

RTC 2-3: We have done our best to work with the manufacturer of diuron and the EPA to obtain additional plant toxicity data. We requested additional plant toxicity data from the manufacturer of diuron, and they submitted several studies to the EPA. We have requested the studies from EPA, but have only received one of the studies at the time of publication of this report, which has been incorporated into the criteria report. When additional studies are available, the data should be evaluated and incorporated into a revised criteria report.

COMMENT 2-4: In accordance with the published method for an acute criterion, the authors divided the lowest LC50 by an assessment factor since acceptable data for only two taxa met the data quality requirements of the method (data for 13 other taxa were classified low reliability, low relevance). As the assessment factors were based on data for neurotoxic insecticides, WPHA believes that the application of the assessment factor to a herbicide with a different mode of action requires a more convincing rationale than is provided in the document.

RTC 2-4: It is stated in the Acute Criterion section of the report that the Assessment Factor approach should be used with caution for an herbicide. However, diuron is a chlorinated compound that does exhibit toxicity to animals and the mechanism is not clear. The AFs given in the methodology (Table 3.13, TenBrook *et al.* 2009a) are the most specific AFs available for organic pesticides, compared to those used in the Great Lakes Initiative (USEPA 2003), which derived AFs from a much broader array of chemicals, such as metals and industrial chemicals.

COMMENT 2-5: In a significant departure from the data quality requirements of the method, the authors applied an additional safety factor of 2 so that the final acute criterion was below all endpoints reported for all taxa regardless of the reliability of the data. WPHA believes it's inappropriate that low reliability, low relevance data dictate the final acute criterion as it appears to contradict the goals of a data quality review. As noted in the document, this resulted in a criterion that was equivalent to the benchmark proposed by the US EPA.

RTC 2-5: See RTC 1-2.

COMMENT 2-6: Aquatic plant endpoints should be based on measurements of growth or growth rate as recommended by the Organisation for Economic Co-operation and Development (OECD) and should consider the potential for recovery. Aquatic plant studies are designed to allow determination of the EC50, which is a conservative, robust endpoint. The endpoints measured in aquatic plant studies are sublethal (effects on growth), and the effects are often reversible. Aquatic plants exposed to diuron at the EC50 recover and resume normal growth when exposed to fresh growth medium. WPHA believes that the No Observed Effect Concentration (NOEC) is not an appropriate endpoint, since it is dependent on dose-selection and cannot be compared among species.

RTC 2-6: The goals of this method include the narrative objective of the Regional Board to maintain water free of "toxic substances in concentrations that produce detrimental physiological responses in plant, animal, or aquatic life," (CVRWQCB 2006). The criteria are designed to be in accordance with the narrative objective of the Central Valley Regional Water Quality Control Board, which is to maintain waters free of "toxic substances in concentrations that produce detrimental physiological responses in plant, animal, or aquatic life" (CVRWQCB 2006). While plants and algae may be able to recover from exposures to diuron, the goal of the derived water quality criteria are to prevent any adverse effects due to exposure. The methodology is designed to derive a concentration at which detrimental effects do not occur, not a concentration at which an organism could recover from detrimental effects. The magnitude component of water quality criteria is designed to be set at a level that will not cause harm to any organisms. The potential for organism recovery after brief exposures to contaminants is addressed by the frequency component of the final criteria statement (section 2-3.4, TenBrook *et al.* 2009). With regards to the use of a NOEC value, see RTC 1-4.

2.3. Comment Letter 3 – Daniel McClure, P.E., Central Valley Regional Water Quality Control Board

COMMENT 3-1: The authors have done a thorough review of the toxicology literature, and applied the UC Davis criteria derivation methodology developed by Tenbrook, et al., in a sound and transparent manner to derive criteria that should be protective of aquatic life.

RTC 3-1: Comment acknowledged.

COMMENT 3-2: For the acute criteria, the preliminary acute criterion of 168 ug/L was higher than the 96-hour LC50 for Gammarus lacustris (160 ug/L) from the supplemental data set. Therefore an additional safety factor

is applied to the preliminary acute criterion to come up with the final acute criterion of 84 ug/L. The toxicity value for Gammarus lacustris was in the supplemental data set since the study it came from did not rate high enough to be in the primary data set used in the direct calculation of criteria. The Gammarus lacustris study did rate high enough to be included in the supplemental data set. It makes sense to consider the toxicity values in the primary data set. Considering values for sensitive species from the supplemental data set is consistent with Section 3-6.1 of the criteria derivation methodology. Absent any fatal flaw or reason to believe that the Gammarus lacustris study is invalid seems to make sense to adjust the criteria to ensure that criteria are protective of Gammarus species. On the other hand there is some uncertainty about the one available Gammarus lacustris study in the supplemental data set. As we have not reviewed the issue in detail, we are not making a recommendation at this time as to whether the evidence about the sensitivity of Gammarus species is sufficient to warrant adjusting the criteria downward. We do recommend that the final report should provide more detail on the potential adjustment of the criteria in response to the Gammarus lacustris study from the supplemental data set.

RTC 3-2: The *Gammarus lacustris* toxicity value is not used to justify downward adjustment of the acute criterion in the final criteria report because the value was based on nominal concentrations, and the methodology specifies that criteria adjustment should be based on measured toxicity values (section 3-6.1, TenBrook *et al.* 2009). The acute criterion is rounded to two significant digits in the final report to be 170 μg/L.

COMMENT 3-3: The Criteria statement indicates that the recommended criteria would be protective of aquatic life in the Sacramento and San Joaquin River Basins. The specificity of these criteria to those basins should not be over-emphasized. It would be useful to note in the criteria statement that these criteria should also likely be protective of aquatic life in freshwater ecosystems in North America, unless species more sensitive than are represented by the species examined in the development of these criteria are likely to occur in those ecosystems.

RTC 3-3: The final criteria statement has been revised to state that these criteria should also be protective of aquatic life in other freshwater ecosystems in North America, which is also stated in the methodology (TenBrook *et al.* 2009) that was used to derive these criteria.

COMMENT 3-4: It would be useful to explicitly discuss the USEPA (1985) methodology and whether adequate data were available to meet the requirements of the USEPA methodology, and, if possible, what criteria derived using the USEPA methodology would likely look like.

RTC 3-4: The criteria report has been amended with a section titled "Comparison to the National Standard Methods," in which the US EPA (1985) criteria derivation methodology was used to calculate acute and chronic criteria with the data set collected using the TenBrook *et al.* (2009) methodology.

> **COMMENT 3-5:** The criteria document compares the derived criteria to the benchmarks developed by the USEPA office of pesticide programs (OPP). It is important to properly qualify these OPP benchmarks. These benchmarks were published by OPP, not USEPA's Office of Water, and they are not "established" as, or intended to be, aquatic life criteria. Therefore in the criteria document, comparison with OPP benchmarks should either be removed or highly qualified. The OPP plant benchmark for diuron is an EC50. Setting a water quality criteria at a level where known toxic effects occur would not be consistent with the level of protection required by the Basin Plan.

RTC 3-5: The report has been revised to clarify that the OPP benchmarks are not equivalent to aquatic life criteria and they cannot be directly compared. OPP's procedures rely, at a minimum, on data for the most sensitive tested effects concentration for each taxon and cannot be used to set water quality standards under the Clean Water Act, unlike water quality criteria.

> **COMMENT 3-6:** The discussion of uncertainty in section 17 should review the following information gaps:
>
> - The genera that would be needed to do a full species sensitivity distribution.
> - The need for a Gammarus species toxicity study that ranked high enough to be included directly in the criteria calculation.
> - The lack of directly applicable information on the synergistic effects of the combination of diuron with other compounds, especially those for which there is an indication of synergistic effects – organophosphate pesticides and fungicides.
> - The need for follow up on the studies discussed in section 12 for species which had toxicity values lower than the derived criteria to see if any of the species or endpoints involved could warrant further lowering the recommended criteria.

RTC 3-6: Section 17 of the report has been revised to discuss and emphasize the above points.

> **COMMENT 3-7:** A table of contents would make the document easier to read.

RTC 3-7: A table of contents has been added to the final report.

COMMENT 3-8: If possible, it would be useful to display the toxicity information in data tables in order of species sensitivity.

RTC 3-8: The toxicity data are displayed in order of species because when there are multiple toxicity values for a species, the geometric mean of those values is calculated to give the final species mean toxicity value. If the data were displayed in order of sensitivity, all of the values for a given species would not be adjacent and the calculation would be less clear, and it would be more difficult to compare the range of toxicity values for a given species.

COMMENT 3-9: We appreciate the tremendous effort that has gone into development of this document and look forward to seeing it finalized.

RTC 3-9: Comment acknowledged.

2.4. Comment Letter 4 – Aldos Barefoot, Ph.D., DuPont Crop Protection

COMMENT 4-1: We recommend that data used in regulatory decision-making processes be conducted in accordance with Good Laboratory Practice (GLP) and in accordance with internationally accepted test guidelines. We support the effort by Fojut *et al.* to use data with high relevance and high reliability and recognize the significant effort undertaken by the authors to evaluate the many reports and literature references available for diuron. We note that the studies selected for derivation of the acute and chronic criteria were studies submitted by DuPont to support registration actions of the US EPA and the State of California.

RTC 4-1: Comment acknowledged.

COMMENT 4-2: As study designs and data quality requirements have changed, DuPont has continued to update the database of ecological effects tests, and we are preparing to submit to the US EPA several studies that are relevant to establishing water quality criteria. These studies will be available to you through a Freedom of Information Act request after EPA has assigned an MRID. The new studies include data on three algal species (Table 1). A recently conducted study (Ferrell, 2006) on *Lemna gibba* has already been submitted to the EPA (MRID 46996701).

Table 1
Algal and Aquatic Plant Studies

Study	Organism	Code/Lab	Report Date	Biomass Endpoint(s)	Growth Rate Endpoint(s)	GLP
Algal Toxicity	*Selenastrum capricornutum*	Douglas & Handley DPT 171	1988	72 hr EC_{50} – 0.018 mg/L 120 hr NOEC - ~0.01 mg/L	0.022 mg/L (120 hrs) 120 hr NOEC - ~0.08 mg/L	Yes
Algal Toxicity	*Synechococcus leopoliensis*	D. Dengler, DuPont-19438	2006a	0.026 mg/L (72 hr) NOEC – 0.0037 mg/L	0.380 mg/L (72 hr) NOEC – 0.011 mg/L	Yes
Algal Toxicity	*Navicula pelliculosa*	D. Dengler, DuPont-19440	2006b	0.022 mg/L (72 hr) NOEC – 0.011 mg/L	0.065 mg/L (72 hr) NOEC – 0.011 mg/L	Yes
Aquatic Plant	*Lemna gibba* G3	B. Ferrell DuPont-20775 MRID 46996701	2006	0.0144 mg/L (7 day EC_{50}) Based on Biomass Yield NOEC – 0.00247 mg/L	0.0203 mg/L (7 day EC_{50}) Based on Biomass NOEC – 0.00247 mg/L	Yes

RTC 4-2: We appreciate the willingness of DuPont to submit additional study data to the US EPA so that it may be requested for use in criteria derivation. We have requested all of the studies listed in Table 1 from the US EPA. As of March 10, 2010, only one of the studies has been received (Ferrell 2006), and it has been evaluated and added to the chronic plant data set. We acknowledge that when the other studies are received the diuron criteria should be reviewed and potentially revised to incorporate the additional data.

> **COMMENT 4-3:** Many of the data reports detailed in the Fojut *et al* diuron review are useful, scientifically valid reports, but these studies typically are not conducted in compliance with GLP standards or internationally accepted test guidelines and do not meet US EPA and OECD standards for data used in regulatory decision-making processes.

RTC 4-3: Very few plant toxicity studies we were able to identify followed standard test guidelines, as evidenced by the very small acceptable (RR) chronic plant data set. We have been worked with DuPont and the EPA to obtain additional plant studies that do follow standard test methods.

> **COMMENT 4-4:** Diuron is algistatic/phytostatic to algae and aquatic plants. That is, after being placed into fresh, diuron-free medium, algae and aquatic plants were found to recover. This was observed in regulatory guideline studies with two sensitive species, *Selenastrum capricornutum* and *Lemna gibba*. In one of the tests with *Selenastrum capricornutum* (Douglas and Handley, 1988), a recovery phase determined that diuron was algistatic at test concentrations up to 0.16 mg/L, the highest concentration tested. In a test with *Lemna gibba* (Ferrell, 2006), a 14-day recovery period followed by a 7-day exposure period determined that recovery (i.e., growth and reproduction) occurred at test concentrations up to 0.0791 mg/L, the highest concentration tested. These recovery values can therefore be identified as the No Observed Adverse Effect Concentrations (NOAEC) for algae and Lemna. Because both algae and aquatic plants were able to recover after an episodic exposure, the recovery should be taken into consideration when determining the chronic water quality criterion.

RTC 4-4: The criteria are designed to be in accordance with the narrative objective of the Central Valley Regional Water Quality Control Board, which is to maintain waters free of “toxic substances in concentrations that produce detrimental physiological responses in plant, animal, or aquatic life" (CVRWQCB 2004). While plants and algae may be able to recover from exposures to diuron, the goal of the derived water quality criteria are to prevent any adverse effects due to exposure. The methodology is designed to derive a concentration at which detrimental effects do not occur, not a concentration at which an organism could recover from detrimental effects. The magnitude component of water quality criteria is designed to be set at a level that will not cause harm to any organisms. The potential for organism recovery after brief exposures to contaminants is addressed by the frequency component of the final criteria statement (section 2-3.4, TenBrook *et al.* 2009).

> **COMMENT 4-5:** The data summaries for the bioaccumulation studies conducted by Isensee (1976) and Call et al (1987) should be included in the appendix. The work by Isensee should not be considered relevant for the diuron criteria derivation and should be removed from Table 1 and Section 13 (Bioaccumulation). Our conclusion is based on the screening level study design as shown by the static test systems, low replication of the test, low number of fish (two), and the determination of the bioconcentration factor based on total radioactive residues rather than residues of diuron. The work by Call *et al* is more representative of a regulatory guideline study design than the Isensee study. Fish were exposed to the test material in a flow-through design (not explicitly indicated in the paper, but a static test system is not possible), ensuring exposure to constant levels of the test material. Residues of diuron in fish were determined during the periods of uptake and depuration. The authors determined that 1.3% of the total tissue radioactivity was diuron, resulting in a mean bioconcentration factor of 2, not log 2. This should be changed in Table 1 and in Section 13. Using a BCF value of 2, the calculations for the mallard and human $NOEC_{water}$ values will change to 2,500,000 µg/L and 1000 µg/L, respectively. These values exceed the proposed chronic criterion by factors of 2,000,000 and 800, respectively.

RTC 4-5: The data summaries for the Call *et al.* (1983, 1977) studies have been added to the appendix. There is not a data summary for the Isensee (1976) study because only single-species toxicity tests are summarized in the data summary sheets. The study by Isensee (1976) is less reliable than the study by Call *et al.*, as described in comment 4-5 (above). Especially of note is that the Call *et al.* test was a flow-through exposure, which is preferred to static exposures, as used by Isensee (1976). Thus, the bioaccumulation potential of mallards and humans in the Bioaccumulation section of the report were re-calculated with the BCF value reported by Call *et al.* (1983, 1987). The BCF value from the Call *et al.* studies has been corrected in Table 1 of the report, as noted in the comment. The

mallard and human $NOEC_{water}$ values have been re-calculated in the Bioaccumulation section (now section 15), with the BCF value from the Call *et al.* studies, which yielded higher values.

> **COMMENT 4-6:** The authors selected data for two taxa as reliable and relevant for establishing an acute water quality criterion. Following the method for an acute criterion, the authors used an assessment factor of 36 to divide the lowest EC_{50} and produce the acute value. The authors discussed the uncertainties in applying an assessment factor based on neurotoxic insecticides to a herbicide, but made no other effort to justify applying the same assessment factor to diuron. A sound rationale for this decision is desirable and should be developed before applying the method to diuron or other herbicides. There are data for 15 species in tables 4 and 5, of which data for 3 species are considered reliable and relevant. The acute value of 333 µg/L calculated through the use of the assessment factor (section 7, page 6) is less than the LC/EC50 for all species with one exception, *Gammarus lacustris* (Sanders, 1969) which the authors categorized as less reliable/less relevant (LL). It is not appropriate to increase the assessment factor when the existing data is not considered adequate for construction of a species sensitivity distribution.

RTC 4-6: See RTC 1-3 for discussion of the use of assessment factors. See RTC 1-2 for discussion of the use of supplemental data to adjust criteria. The acute criterion is rounded to two significant digits and is reported as 170 mg/L in the final criteria report because the *Gammarus lacustris* study used nominal concentrations, which cannot be used to adjust criteria according to the methodology (section 3-6.1, TenBrook *et al.* 2009a).

> **COMMENT 4-7:** The recommended acute criterion (Section 7) according to the method of Tenbrook *et al.,* 2009 should be 168 µg/L. The addition of another assessment factor of two based on a study identified as unreliable by the authors is not appropriate. The study by Sanders (1969) should not be considered in this assessment since there is no data for the controls. Without this data, it is impossible to determine the overall health of the test organisms used in the study. Table 6 clearly identifies this study as 'LL' because the study design was not based on a standard study design and a control response was not reported. Applying an additional safety factor so that the final acute criterion was below all endpoints reported for all taxa appears to negate the value of the data review for identifying data that is reliable for establishing a water quality criterion. The final criterion, using the additional, arbitrary assessment factor was coincidentally equal to the US EPA benchmark value of 80 µg/L and was accepted because of the similarity to the EPA value rather than as a result of the criteria outlined in Tenbrook *et al*. 2009. The revision of the acute criterion to 168 µg/L should be reflected in the appropriate portion of Section 18, Final criteria statement.

RTC 4-7: The method clearly states that criteria may be adjusted downward to be protective of sensitive species, but that it should be based on data rated RR, RL, LR, or LL that used measured concentrations (section 3-6.1, TenBrook *et al.* 2009). The acute criterion is rounded to two significant digits and is reported as 170 mg/L in the final criteria report because the *Gammarus lacustris* study used nominal concentrations, which cannot be used to adjust criteria according to the methodology (section 3-6.1, TenBrook *et al.* 2009a).

> **COMMENT 4-8:** In Tenbrook *et al*., 2009, Chapter 2 (Evaluation and Selection Methods), Section 2-2.1.2 (Hypothesis tests vs. regression analysis) "…the MATC is the value used in the new methodology to calculate the chronic criterion." Following this guidance, the chronic criterion (Section 8) should be 1.8 µg/L, the MATC from Blasberg et al. 1991.

RTC 4-8: The procedure for derivation of a chronic criterion for an herbicide specifies that when there are highly rated MATC values for at least five different plant species, a SSD should be fit to the plant data (section 3-4.3, TenBrook *et al.* 2009). When there are less than five MATC values for plant species, then the methodology specifies that the chronic criterion will be equal to the lowest NOEC for an important plant species. The NOEC is used when the criterion is based on only one toxicity value in order to be conservative, considering how much uncertainty is involved when a criterion is calculated with only one value.

> **COMMENT 4-9:** Data is available in Blasberg *et al*. to calculate the EC50, and Tenbrook *et al*. state in Chapter 3, Section 2.1.1.2 that an ECx may be used for criteria development. Aquatic plant studies are designed to allow determination of the EC50, which is a conservative, robust endpoint. The endpoints measured in aquatic plant studies are sublethal (effects on growth), and the effects are generally reversible. Because algal and aquatic plant studies are based on effects such as population growth rate and not on individual effects such as mortality, the EC50 is an appropriate endpoint for establishing a water quality criterion. The NOEC is not an appropriate endpoint, since it is dependent on dose-selection and cannot be compared among species.

RTC 4-9: EC_x values may be used to calculate chronic criteria when there is information in a study to clearly indicate what level of *x* is representative of a no-effect level for that particular species. The Blasberg *et al.* (1991) study does not demonstrate which level of *x* can be considered a no-effect level, and therefore the EC_{50} value reported in that study cannot be used to calculate the chronic criterion. A 50% effect on an organism cannot be considered a no-effect level, and therefore an EC_{50} is not a reasonable level at which to set the chronic

criterion, which is designed to protect organisms from sublethal effects. NOEC values are evaluated to determine if they are reasonable approximations of no-effect levels in the data evaluation process (section 2-2.1.2, TenBrook *et al.* 2009).

> **COMMENT 4-10:** Aquatic plant endpoints should be based on measurements of growth or growth rate as recommended by OECD and should consider the potential for recovery. We recommend that the Central Valley Water Quality Control Board select the EC50 based on growth rate instead of the NOEC to take account of the type of effects measured in aquatic plant studies. The potential for recovery was not considered by Fojut *et al*., but should be the basis for determining a chronic water quality criterion since the exposures to diuron will be episodic. This change should be reflected in Section 18, Final criteria statement.

RTC 4-10: The frequency component of the final criteria statement, rather than the magnitude component, takes into account potential recovery of organisms after brief and episodic exposures to contaminants. As addressed in RTC 4-9, an EC_{50} level is not an appropriate level to set the magnitude component because effects on 50% of organisms cannot be considered a no-effect level. The magnitude component is designed to be set at a level that will not cause any detrimental effects, without consideration of the potential for recovery.

2.5. Comment Letter 5 – Stephen L. Clark and R. Scott Ogle, Ph.D., Pacific EcoRisk

> **COMMENT 5-1:** Although the public comment window for the "Phase II" Report (TenBrook et al., 2009) method has passed, we are compelled to express that this method (and others) lack an effective "kill switch" to outright reject critically flawed studies for use in deriving water quality criteria. There are two critical elements that Pacific EcoRisk believes should be part of any credible scientific publication that is used for criteria derivations:
>
> - Measured (i.e., verified analytically) concentrations of the chemical being tested; and
> - A valid control response (i.e., a minimally acceptable level of test response such as 90% survival in an acute test) for the given test method.
>
> Studies that only report "nominal" test concentrations should not be used for criteria derivation. There is simply no assurance that the concentrations that are reported in such literature sources are the **actual** concentrations for the exposures, particularly for older studies in which the

ancillary QA measures that are standard for modern studies were not performed. Errors in solution preparation, accuracy of the analytical equipment/instrumentation (e.g., balances, pipettes, etc.), chemical stability, chemical solubility, etc., can potentially result in significant differences between the nominal concentration and the actual concentration of the exposure. Furthermore, the statistical analysis of the test data should be performed using the actual measured concentrations to be accurate. Without such assurance of accuracy, the applicability of such test results for criteria derivation is questionable and could result in incorrect criteria (e.g., both under protective or over protective).

The Phase II Report rating system allows the use of a study that is complete in all aspects except that the Control treatment "failed", since this would result in a score of 92.5 out of 100 for the study. Under most regulatory applications (e.g., CVRWQCB NPDES permits), and for most aquatic toxicity test methods (e.g., EPA aquatic toxicity test methods), a failed Control treatment response invalidates the test, with a concomitant requirement to repeat the test. It is inconsistent for one regulatory program (e.g., NPDES) to invalidate data based on a failed Control treatment response, but for another regulatory framework (e.g., the Basin Plan amendment process) to accept literature-derived test results with a failed Control treatment response for use in deriving water quality criteria. Although the above comments relate primarily to the method established in "Phase II" Report (TenBrook et al., 2009), the absence of "kill switches" in the method has allowed the use of questionable? data in the Diuron Criteria Derivation.

RTC 5-1: The use of nominal concentrations does not exclude data from use in criteria derivation, but data used to adjust criteria after the initial derivation must use measured concentrations (3-6.1, TenBrook *et al.* 2009). The data evaluation process of the methodology has been thoroughly reviewed by both peer review and public comment processes, but may be revised in the future.

COMMENT 5-2: Unilateral selection of some but not all of the scoring methods in the Phase II Report is inappropriate. As per Section 14.10.2.3.1 of Method 1003.0 (the green alga, Selenastrum capricornutum, growth test, EPA 821-R-02-013): "Alkalinity, hardness, and conductivity are measured at the beginning of the test in the high, medium, and low concentrations and control before they are dispensed to the test chambers…" Clearly, EPA scientists have deemed that these parameters are an essential component of the Selenastrum method. Awarding full points to studies for which essential water quality data (e.g., alkalinity, hardness, and conductivity) are not available is not only unacceptable by the EPA method, but it also creates an undesirable slippery slope of selective adherence of the Phase II protocol for this criteria derivation and for future criteria derivations.

The Diuron Criteria Derivation should be revised to award properly diminished points to the studies that lack critical support data.

RTC 5-2: We have re-rated the studies in which some water quality parameters were omitted from scoring, which is reflected in changes to the data tables. Data summary sheets are available for every study in the Appendix with complete scoring for each study at the bottom of the summary sheet.

The USEPA (2002) algal test method cited above (Section 14.10.2.3.1 of Method 1003.0, the green alga, Selenastrum capricornutum, growth test, EPA 821-R-02-013) is used for toxicity tests with receiving and effluent waters, not for toxicity testing with a pure compound in clean media. Valid standard methods for algal toxicity testing (e.g., 40 CFR 797.1050 Algal Acute Toxicity Test (USEPA 1996), ASTM E1218 (2004)), do not call for the measurement of hardness, alkalinity, dissolved oxygen, or conductivity. These test methods call for the use of a nutrient growth media prepared according to ASTM standards as the dilution water, which are described in ASTM E1218 (2004).

COMMENT 5-3: The derivation authors' unilateral decision to deviate from the Phase II Report protocol in implementing a second application of a safety factor is unwarranted and inappropriate without justification that is consistent with the Phase II Report protocol. If this approach is allowed to move forward, a precedent is being established by which the authors of any other future pesticide criteria derivations may elect to deviate from the Phase II protocol at will and without justification.

RTC 5-3: In the final diuron criteria report, the acute criterion is not adjusted downward based on the sensitive species data because the *Gammarus lacustris* data that is below the derived criterion used nominal concentrations, and measured concentrations are required by the methodology for criteria adjustment. However, the application of an additional safety factor is not a deviation for the methodology. As described in the method, "criteria must be protective of aquatic life, and therefore must err on the side of conservatism when data are lacking," (section 2-3.2.1, TenBrook *et al.* 2009a). The methodology states that if a calculated criterion is higher than toxicity values reported for a particularly sensitive species, then the criterion may require downward adjustment, if the data used measured concentrations (section 3-6.1, TenBrook *et al.* 2009). When a SSD is used to calculate the criterion, a lower distributional estimate is used for downward adjustment. Because an AF was used for the acute criterion calculation for diuron, instead of a SSD, an additional safety factor could be applied for downward adjustment of the criterion, if measured data was available that showed the criterion was underprotective of a sensitive species.

COMMENT 5-4: The authors of the derivation acknowledge that the Sanders 1969 paper was rated "Less reliable-Less reliable" (LL), and that Sanders did not report the test response for the Control treatment of their test. As per our Comment #1 about the need for "kill switches" for data evaluations, we suggest that this paper should be rejected for use in criteria derivation since it is critically flawed. This paper should by no means be used to justify the application of an additional safety factor.

RTC 5-4: The proposed acute criterion of 170 μg/L is compared to the acceptable and supplemental data sets as per instruction in the methodology (sections 2-5.1 and 3-6.1, TenBrook *et al.* 2009). The Sanders (1969) study is a part of the supplemental data set, as evidenced by its rating of LL (Table 6), and therefore can be used for this comparison, but cannot be used for downward adjustment, because the study used nominal concentrations (section 3-6.1, TenBrook *et al.* 2009). The acute criterion is not adjusted downward in the final report.

COMMENT 5-5: As per PER Comment 1 above, papers without measured pesticide concentrations are severely flawed and should not be used for criteria derivation. As this would result in the rejection of all of the literature-reported data that the authors used in their derivation (reported in Table 7a), we suggest the following:

1. The current derivation effort identify that there are no acceptable chronic data and that a chronic criterion can not be established at this time;
2. That in the absence of appropriate data, a chronic criterion be established but that it be identified as an "Interim Chronic Criterion" to be used until such time as appropriate data become available;
3. That scientifically sound studies should be performed to support the chronic criteria derivation rather than using flawed studies to simply obtain a number.

RTC 5-5: There was a reporting error in the final chronic plant data set (Table 7a), and the toxicity values for *Pseudokirchneriella subcapitata* reported by Blasberg *et al.* 1991 were actually calculated based on measured toxicity values, not nominal as reported in the draft report. Toxicity values from tests with measured concentrations are preferred, but the use of nominal concentrations does not prevent toxicity values from being used in criteria derivation (sections 2-2.7 and 3-2.4, TenBrook *et al.* 2009).

COMMENT 5-6: The NOEC is a measure of toxicity that is often used for regulatory purposes (i.e., calculation of Toxic Units [TU], where TU = 100/NOEC). Determination of the NOEC is based upon statistical comparisons of test treatments with a Control treatment to determine if there is a statistically significant reduction at the test treatment relative to the Control. Recognized problems with the use of the NOEC as a

regulatory benchmark or for use in the derivation of water quality criteria include:

1. The typical toxicity test consists of the evaluation of 5 or 6 specific chemical concentrations that are generally arbitrarily decided upon (e.g., the a priori decision to use 5 ug/L, 10 ug/L, 25 ug/L, 50 ug/L, and 100 ug/L of the chemical in question as the test treatments). As a result, and by definition, the NOEC will almost never accurately identify the actual chemical concentration at which there is “no effect”, but rather will be limited to the identification of the highest test treatment at which there is no effect. For instance, in the example test concentrations described above, it would be possible to have a slight but statistically significant effect at the 100 ug/L concentration for a chemical that would have no significant effect at the 90 ug/L concentration. However, since the next highest test treatment is 50 ug/L, the NOEC will be 50 ug/L, and not the true no effect concentration of 90 ug/L.

In contrast, point estimates (e.g., the Effect Concentration (EC) and Inhibition Concentration (IC) point estimates) are empirically-derived estimates of the actual test concentration at which some magnitude of response occurs. For instance, the algal IC25 would be the test concentration at which there is expected to be a 25% reduction in algal cell density. The EC25 and IC25 can therefore be used to establish a regulatory limit based upon the degree of response that is determined to be acceptable by the regulatory agency.

2. The potential NOECs are limited to the test concentrations being tested. If the test concentrations are not specified, then the concentrations used in various studies may differ, hence resulting in different NOECs due strictly to lab practices.

In contrast, the EC and IC point estimates are independent of the test concentrations used.

3. The statistical methods for determining NOECs are limited to using only the data for the Control treatment and the test treatments in question. None of the other test data are used in that statistical comparison. As result, none of the other relevant test data information that help characterize concentration-response, etc., are being used.

In contrast, the calculation of the EC and IC point estimate use all of the test data to empirically model the concentration-response curve from which the point estimates are derived.

4. The statistical calculation of the NOEC is strongly determined by the inter-replicate variability that is achieved by the testing lab. Statistical power (i.e., the ability to detect “significant” differences between test treatments) is a direct function of inter-replicate variability: the lower the variability, the more powerful the statistics, and the greater ability to identify an increasingly smaller difference between treatments as being “significant”. As a result, for a given

test media, the NOEC could be expected to vary from lab to lab (or from test to test), depending upon each lab's ability to achieve precision in each test.

In contrast, the role of inter-replicate variability in concentration-response modeling is limited to the determination of the confidence limits - the determination of an EC or IC point estimate is relatively independent of inter-replicate variability.

The NOEC is a statistical benchmark that is easy to calculate and easy to understand, and it has a long history of regulatory usage for just these reasons. However, many scientists agree that there are serious problems with usage of NOECs in interpretation of toxicity tests (and therefore in the use of NOECs for criteria derivation), and that a regression-based approach such as used in the EC and IC point estimation approach is a better alternative. Indeed, regulatory programs that have conducted serious workshops and overhauls of their statistical methodologies have abandoned the NOEC and have adopted the regression-based approach (OECD 1998). Since the EC50 is available for the Blasburg et al., 1991 paper that the derivation authors are basing their proposed chronic criterion, this suggests that other point estimates would be available for the data. We encourage the authors consider using a more accurate point estimate for the diuron chronic criteria derivation rather than the NOEC.

RTC 5-6: The Phase II methodology recognizes the limitations of hypothesis test data, and chronic data expressed as results of hypothesis tests are evaluated to ensure that the reported toxicity values are reasonable estimates of no-effect levels (section 2.1.2, TenBrook *et al.* 2009). Because the goal of the method is to prevent detrimental effects to organisms, an EC_{50} is not a valid toxicity value for use in derivation of a chronic criterion because a 50% reduction compared to the control cannot be considered "no effect." If a study were available that demonstrated what level of *x* represented a no-effect level, then an EC_x toxicity value could be used in chronic criterion calculation (section 2-2.1.2, TenBrook *et al.* 2009).

3.0 Response to Comment to Peer Reviews

3.1. Peer Review 1 – John P. Knezovich, Ph.D., UC-Davis, Lawrence Livermore National Laboratory

REVIEW 1-1: *Overview*

The freshwater criteria for diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) defined in this draft report was derived using methodology recently developed

by Tenbrook *et al*. (2009)[1]. The methodology considers relevance of the endpoints and quality of the data in derivation of the criteria. This methodology was motivated by the California Regional Water Quality Control Board's desire to employ rigorous methods to develop criteria for protection of the Sacramento and San Joaquin River Watershed.

Response to review (RTR) 1-1: Comment acknowledged.

Review 1-2: The report provides a comprehensive summary of the physical-chemical data for diuron. This data set is straightforward and indicates that this herbicide has moderate solubility, low volatility, moderate ability to bioaccumulate, and is somewhat persistent in aqueous environments (i.e., relatively low rates of hydrolysis, photolysis, and biodegradation). Accordingly, this herbicide's physical-chemical characteristics make its exposure to aquatic organisms a relevant concern.

RTR 1-2: Comment acknowledged.

Review 1-3: The authors evaluated 84 published studies of diuron toxicity to develop the proposed criteria. Relevance was determined using the aforementioned criteria[1] and data for studies that were deemed acceptable were evaluated. Adequate and reliable data is available for determining acute toxicity using animal studies. However, aquatic plant toxicity data that is critical for assessing the potential ecological hazard posed by diuron is more difficult to evaluate because standard endpoints are generally lacking and exposure durations are highly variable. To constrain this evaluation, only data for tests that lasted at least 24 hours were used for criteria development, which is in accordance with standard methods.

RTR 1-3: Comment acknowledged.

Review 1-4: *Acute criterion*
The acute criterion for diuron was calculated using the Assessment Factor procedure, which was developed for application to neurotoxic insecticides. Because diuron is an herbicide with unknown mechanisms of toxicity in animals, the authors state that this approach will be used "with caution" for this herbicide. The majority of available data was excluded from the final derivation for legitimate reasons (e.g., lack of controls, use of saltwater species). The large number of exclusions resulted in data for two required taxa (i.e., salmonid fish, and insects) not being used. Although these

[1] P. Tenbrook *et al*. (2009). *Methodology for derivation of pesticide water quality criteria for the protection of aquatic life in the Sacramento and San Joaquin River basins. Phase II: Methodology development and derivation of chlorpyrifos criteria.* Report prepared for the Central Valley Regional Water Quality Control Board, Rancho Cordova, CA.

exclusions were applied appropriately, much of the excluded data includes LC/EC_{50} values that are relatively low. Accordingly, the derived criterion should be re-evaluated if data of sufficient quality for the missing taxa becomes available.

RTR 1-4: Comment acknowledged.

Review 1-5: The lowest acceptable acute LC_{50} value of 12 mg/L for *Daphnia magna* was used to derive the acute criterion. This value was appropriately divided by the assessment factor of 36, which takes into account the species sensitivity distribution. The resulting acute value of 0.3333 mg/L was divided by a safety factor of 2 to yield a preliminary acute criterion value of 0.1667 mg/L, which was rounded to 168 µg/L (this value was not rounded correctly and should be 167 µg/L). Because the animal data set was relatively sparse, an additional safety factor of 2 was applied to the preliminary acute criterion to yield a recommended criterion of 84 µg/L. This additional safety factor is consistent with EPA's methodology for derivation of an acute value for diuron, which yields a benchmark of 80 µg/L. It also appears to reflect the call for caution indicated by the authors in the use of the Assessment Factor procedure.

RTR 1-5: The initial acute criterion value has been revised so that it is rounded to two significant digits to be 170 µg/L. The acute criterion is no longer adjusted downward to 84 µg/L, because the data for *Gammarus lacustris* used to justify downward adjustment used nominal concentrations, and the methodology requires that data for sensitive species used to adjust the criteria must use measured concentrations (section 3-6.1, TenBrook *et al.* 2009).

The goal of the criteria derivation methodology used in this report is not to match the numeric criteria, objectives or benchmarks used by other agencies, but to derive protective criteria for aquatic life.

Review 1-6: *Chronic criterion*
The chronic criterion was derived by assessing endpoints for plant studies that were linked to survival, growth or reproduction. In general, the lack of consistent study protocols and toxicity endpoints confounds the derivation of a robust chronic criterion. This is unfortunate, as the fact that diuron is an herbicide places a high concern over its potential impact on non-target plant species. Only four studies, which evaluated four plant species, provided data that was of high enough quality for inclusion in the final data set. Of these four studies, only one reported an NOEC value for diuron in green algae (i.e., 1.3 µg/L). This value is proposed as the chronic criterion because the methodology calls for adoption of the lowest NOEC value. While this approach adheres to the accepted methodology, it results in a value that has low integrity due to the lack of comparable studies that could provide validation of this number. The authors need to

provide caveats for this criterion that make it clear that it is based on the absolute minimum of data. This is particularly important as several studies that apply non-standard methods indicate that diuron may exhibit toxicity to plants at levels well below the proposed chronic criterion. Although these studies were excluded for legitimate reasons, they do indicate that the chronic criterion for diuron will require continuing evaluation as additional data becomes available.

RTR 1-6: The report has been revised to emphasize that the chronic criterion was derived with the absolute minimum of data, and that is should be re-evaluated as additional data becomes available (sections 17 and 19 of the report). The intent of this method is to use as much data is available to derive criteria, and the lack of relevant and reliable plant data for diuron is the greatest limitation for robust criterion calculation.

There are values below the chronic criterion in the data set, but these studies either tested endpoints that are not clearly linked to survival, growth, or reproduction, used very short exposure durations that do not follow standard methods, were for saltwater organisms, used low purity diuron formulations, or had poorly designed hypothesis tests that were not possible to evaluate due to lack of reporting of data analysis techniques. We agree that as additional quality data becomes available, the criteria should be re-evaluated.

Review 1-7: *Bioavailability*
Data is available that indicates that diuron's availability to aquatic species will be reduced by its interaction (sorption) to black carbon. This is consistent with diuron's organic carbon sorption coefficient (K_{oc}) and with the behavior of other neutral organic compounds. The authors should mention that dissolved organic compounds (DOC) in general as well as clays are likely to inhibit the bioavailability of diuron in natural waters. Because bioavailability is a function of site-specific characteristics of the water body, the authors are correct in concluding that the proposed criteria should be based on whole water concentrations as this is a conservative and appropriate approach.

RTR 1-7: The bioavailability section has been revised to indicate that DOC and clays are likely to inhibit bioavailability of diuron in a similar manner as black carbon, although investigations of these phenomena have not been identified in the literature.

Review 1-8: *Mixtures*
Significant data exists on the influence of chemical mixtures on the toxicity of diuron. Evidence exists that diuron toxicity can be additive, synergistic or weakly antagonistic with compounds and chemicals with which it is likely to co-occur. Because an accurate method to predict the effects of

diuron in the presence of other chemicals does not exist, the authors have advocated that its toxicity should be predicted by the additive concentration method when other similar (i.e., photosystem II) herbicides are present. This is a reasonable and conservative approach that is supported by the literature.

RTR 1-8: Comment acknowledged.

Review 1-9: *Temperature, pH effects*
There is no evidence to support temperature or pH effects on diuron toxicity nor is there reason to expect that these parameters would significantly influence this compound's bioavailability or toxicity.

RTR 1-9: Comment acknowledged.

Review 1-10: *Sensitive species*
The derived acute criterion, which is based on evaluations of animal toxicity, would appear to protect the most sensitive species (i.e., *Gammarus lacustris*) for which there is data. Although data for this species was not used due to low reliability, the proposed criterion of 84 µg/L is approximately one-half of its 96-h LC_{50}. The authors state that the proposed criterion should provide protection for aquatic invertebrates, which appears to be true based on the majority of the (excluded) data set. However, the 96-h LC_{50} for *G. lacustris* presents a cause for concern. A more detailed justification for the exclusion of this data is necessary to strengthen the argument that this data may not be reliable.

RTR 1-10: The toxicity data summary for the *G. lacustris* study has been added to the appendix so that all of the study parameters reported and missing are available in the report. The primary reason for this low relevance rating of the Sanders (1969) study was that they did not report the use of controls or the control responses. Other study details were also not reported, which led to the less reliable rating. This study was conducted in a government lab, and it is likely that controls were tested and a standard method was followed, but we could not confirm this information, and therefore did not use the study for direct criteria derivation. We did not use this data for criteria adjustment in the final report either because they used nominal concentrations, and measured concentrations are required for criteria adjustment by the methodology.

Review 1-11: The chronic criterion of 1.3 µg/L is based on the data obtained from a single study of one species (see *Chronic criterion* above). However, several relatively recent studies report toxicity value well below this criterion. While these studies were largely excluded from consideration for valid reasons, they do provide a strong indication that toxicity in plants may be manifested below 1/3 µg/L. For example, Podola

and Melkonian (2005)[2] used a novel chip system for measuring chlorophyll fluorescence as a response of microalgae to diuron and other herbicide exposures. The authors should provide more detail as to why this study was excluded as it is not clear that the endpoint is nonstandard and does not provide a clear link to adverse effects. Given the high importance of plant toxicity to potential environmental risks posed by diuron, more discussion on the basis for the exclusion of studies that employed non-standard methods is warranted.

RTR 1-11: The Sensitive Species section of the report has been revised to add more detailed explanation of why the studies in the supplemental data set with toxicity values lower than the chronic criterion were not used to adjust the criterion downward. With regards to the study by Podola and Melkonian (2005), chlorophyll fluorescence can be a valid endpoint for plant studies, but the exposure duration in that study was 20 min. The minimum exposure duration used in standard test methods for plants is 24 h. The authors of that study designed their biosensor tests to detect and identify herbicides, and do not discuss how the effects exhibited after this very short exposure duration demonstrate detrimental effects to growth or reproduction.

Review 1-12: *Bioaccumulation*
Diuron has a moderate K_{ow} and therefore a relatively low potential to bioaccumulate in aquatic organisms. Reported bioconcentration factors are consistent with this K_{ow}. The authors conclude that there is "little evidence to show that diuron is a bioaccumulation threat in the environment." The use of the term "threat" here is ill advised as bioaccumulation of diuron, while low, does occur. The threat posed by this compound will be determined if the body burden in an organism reaches a toxic level. It will be more appropriate to simply state that diuron has a relatively low bioaccumulation potential.

RTR 1-12: The Bioaccumulation section of the report has been revised to not use the term "threat" with regards to the bioaccumulative potential of diuron.

Review 1-13: The potential risks posed by food-chain transfer of diuron from aquatic ecosystems was determined by calculating the water concentration that would be required to produce a dietary exposure of 5,000 mg/kg, which is the LD_{50} for mallard ducks. This calculation appears to have been performed incorrectly. The correct concentration is 17.4 mg/L (not 173 mg/L), which is still significantly greater than the expected NOEC and would not be likely to cause adverse impacts on terrestrial wildlife.

[2] B. Podola and M. Melkonian (2005). Selective real-time herbicide monitoring by an array chip biosensor employing diverse microalgae. *J. Applied Phycology*, **17**: 261-271.

RTR 1-13: The calculation results in this section have been revised and corrected.

> **Review 1-14:** A similar calculation for bioaccumulation in finfish, which have a pesticide tolerance level of 2.0 mg/kg for farm-raised fish, yields a water concentration of 7 µg/L. This value is above, but close, to the chronic criteria. The authors are correct to point out that this may be an area that requires additional review to fully consider implications for human health.

RTR 1-14: Comment acknowledged.

> **Review 1-15:** *Ecosystem and other studies*
> The authors reviewed several studies that evaluated potential ecosystem impacts of diuron in microcosms and field work. Many of these studies evaluated impacts on microbial and algal community structures. While the majority of the studies reported effects at levels of diuron that were higher than the proposed chronic value, there is some indication from recent studies that lower levels can influence biofilm community structure. The ecological significance (if any) of this effect is unknown at this time. The overall conclusion of the authors is that the proposed acute and chronic criteria would protect aquatic organisms. While the available data supports this conclusion, additional study of subtle effects of diuron on microbial and algal community structures may warrant re-examination of these criteria at a future time.

RTR 1-15: Comment acknowledged.

> **Review 1-16:** *Harmonization with air and sediment criteria*
> Sediment and air quality standards for diuron do not exist. Partitioning into the water column can serve as a proxy for sediment burdens.

RTR 1-16: Comment acknowledged.

> **Review 1-17:** *Limitations, assumptions, and uncertainties*
> The authors correctly point out that the major source of uncertainty in this evaluation stems from the general paucity of viable data on diuron toxicity, particularly in plant species. Data from published studies was not sufficient to enable the calculation of confidence intervals.

RTR 1-17: Comment acknowledged.

> **Review 1-18:** *Final criteria statement*
> The derived acute and chronic criteria were compared to EPA Benchmarks. The derived acute value is essentially the same as the EPA value (84- vs. 80-µg/l). The chronic value is lower than the EPA value

(1.3- vs. 2.4-µg/L) as a result of this study using the NOEC value instead of the EC_{50} value. The author's justification that the NOEC value would be protective of non-vascular plants is legitimate.

The authors point out that plant toxicity values that are lower than the proposed chronic criterion appear in several publications. Lack of reliability and relevance is cited as the principal reasons for not including data from these studies in this assessment. While this appears to be true at this point in time, plant data is essential to the development of rigorous and adequately protective criteria. The authors' recommendation that the criteria be updated as more reliable plant data becomes available is sound.

RTR 1-18: In the final criteria report the acute criterion is 170 µg/L, which is approximately a factor of 2 higher than the USEPA acute invertebrate benchmark.

Review 1-19: *Errata*
The following typographical errors should be corrected in the final version of the report:

1. Page 7, line 4: "diving" should be "dividing."
2. Page 11, line 12: "30 d old fathead minnows" should be "30-d old fathead minnows."
3. Page 11, lines 24 and 29: "LC_{50}" should be "LD_{50}" (this is a dietary dose reported as mg/kg, not a water-based exposure).

RTR 1-19: The errata are addressed as follows:

1. This typo has been corrected.
2. This typo has been corrected.
3. This toxicity value is correctly reported as an LC_{50} because it is a concentration in feed, not simply a dose of pure diuron. It is reported as an LC_{50} in the original study as well.

3.2. Peer Review 2 – Stella McMillan, Ph.D., California Department of Fish and Game

REVIEW 2-1: Your proposed acute and chronic criteria are 84 and 1.3 µg/L, respectively.

The chronic criterion was derived using four tests on algae and plants. As plants and algae tend to be more sensitive to herbicides, this is appropriate. The final chronic criterion proposed is 1.3 µg/L.

RTR 2-1: In the final criteria report, the acute and chronic criteria are 170 and 1.3 µg/L, respectively.

REVIEW 2-2: The acute criterion was derived using only animal studies because "all plant studies were considered chronic because the typical endpoints of growth or reproduction are inherently chronic". Although this is technically true, as a general rule animals are not as sensitive to herbicides as are plants and algae. It is reasonable to assume that an acute or short-term exposure to diuron would have significant impacts on algae growth. This needs to be addressed in the development of acute criteria. As toxicity tests measuring toxicity to algae are the only tools available, it would be prudent to use these values to generate both acute and chronic criteria. The chronic criterion of 1.3 μg/L could also be used as the acute criterion to protect fish and wildlife.

RTR 2-2: While the available data suggests that plants are much more sensitive to diuron than animals, plant data is not appropriate for derivation of an acute criterion (section 2-2.1.1, TenBrook *et al.* 2009). ASTM (2004) guidelines state that algal tests of short duration (24-120 h) should not be viewed as acute data because they cover multiple generations of algae.

3.3. Peer Review 3 – Xin Deng, Ph.D., California Department of Pesticide Regulation

REVIEW 3-1:The diuron water quality criteria were derived by applying a new methodology recently developed by the University of California, Davis. A total of 84 references including peer reviewed papers and unpublished reports for diuron aquatic toxicity were identified in the report. Following a thorough evaluation on data reliability and relevance, three acute and four chronic toxicity datasets were selected for criteria derivation. Based on the limited data sets and the new method of criteria derivation, the lowest toxicity values from the most sensitive species were used to derive the acute and chronic criteria. In general, the data analysis appears complete and scientifically sound. The resultant chronic criterion is likely protective of aquatic organisms.

RTR 3-1: Comment acknowledged.

REVIEW 3-2:The acute criterion is derived from a 48-h *Daphnia magna* LC50 value that was originally calculated from nominal, total formulation (80 percent active ingredient) concentrations (Baer 1991). As indicated in the report (Appendix A-8), Baer (1991) also reported the measured concentrations accounted for 8-76 percent of the nominal concentrations. This brings up an issue on whether nominal or analytical concentrations should be used for criteria derivation when both are available. Apparently, if a LC50 value calculated from measured concentrations was used, it will result in a significantly lower criterion. Thus, it may be necessary to clarify

this in the methodology. The clarification would be helpful for others to apply the methodology in the future when similar situation is encountered.

RTR 3-2: Measured concentrations are always preferred over nominal concentrations for criteria derivation (sections 2-2.7 and 3-2.4, TenBrook *et al.* 2009), and if raw data is available, toxicity value can be calculated (section 3-2.1.1.2, TenBrook *et al.* 2009). In the Baer (1991) study, only half of the test solution replicates were measured. Without the analytical measured concentrations for all replicates, the raw immobility data cannot be used to re-calculate the toxicity values with the measured concentrations. In this particular study, raw data was only available for half of the replicates, and therefore, toxicity values were not recalculated based on measured concentrations.

REVIEW 3-3: To generate the acute criterion, an additional assessment factor was introduced based on a toxicity test for scud *Gammarus lacustris* (Sanders 1969). As described in the report, the study was less reliable and less relevant because it failed to report responses in the control group and lacked other study details. But this study presented the lowest acute toxicity value among all the studies. Therefore, an additional assessment factor of two was applied in order to generate a value protective of all the aquatic life. However, I would like to point out that the report did not indicate whether replicates had been used, so use of those data need to be better justified. Since this study (Sanders 1969) is critical in deriving the criterion and the data source is not readily available, it may be helpful to present its data evaluation summary in the appendix with other studies that were rated as reliable and relevant.

RTR 3-3: The toxicity data summary for the Sanders (1969) study has been added to the Appendix so that all of the study details and reasons for the score and rating are available and transparent. This study was not used for criteria adjustment in the final criteria report because they used nominal concentrations, and the methodology requires that data used for criteria adjustment is based on measured concentrations (section 3-6.1, TenBrook *et al.* 2009).

REVIEW 3-4: It is worth noting that the value of acute water quality criteria derived from the new methodology is intended to protect animals not plants, because “all the plant and algae toxicity data will be considered chronic toxicity data” (Phase II report, pages 2 through 4).

RTR 3-4: The report has been revised to emphasize that the acute criterion is only for the protection of animals, whereas the chronic criterion is for the protection of plants.

4.0 References

ASTM E1218. 2004. Standard Guide for Conducting Static Toxicity Tests with Microalgae. In: *ASTM E1218 (Environmental Toxicology Standards)*. American Society for Testing and Materials.

Baer KN. 1991. Static, Acute 48-hour EC50 of DPX-14740-165 (Karmex DF) to *Daphnia magna*. United States Environmental Protection Agency report, EPA MRID 420460-03.

Blasberg J, Hicks SL, Bucksaath J. 1991. Acute Toxicity of Diuron to *Selenastrum capricornutum* Printz. United States Environmental Protection Agency report, MRID 422184-01.

Call DJ, Brooke LT, Kent RJ. 1983. Toxicity, Bioconcentration and Metabolism of 5 Herbicides in Freshwater Fish. United States Environmental Protection Agency report, EPA MRID 00141636/TRID 452601029.

Call DJ, Brooke LT, Kent RJ, Knuth ML, Poirier SH, Huot JM, Lima AR. 1987. Bromacil and Diuron Herbicides - Toxicity, Uptake, and Elimination in Freshwater Fish. *Archives of Environmental Contamination and Toxicology*, 16, 607-613.

CVRWQCB. 2006. Sacramento and San Joaquin River Watersheds Pesticide Basin Plan Amendment Fact Sheet. Central Valley Regional Water Quality Control Board, Rancho Cordova, CA.

Douglas MT, Handley JW. 1988. The algistatic activity of diuron technical, DPT 171; DuPont de Nemours France, S. A. Unpublished report.

Ferrell BD. 2006. Diuron (DPX-14740) technical: Static, 7-day growth inhibition toxicity test with *Lemna gibba* G3. DuPont Haskell Laboratory for Health and Environmental Sciences. Newark, DE. MRID 46996701.

Isensee AR. 1976. Variability of Aquatic Model Ecosystem-Derived Data. *International Journal of Environmental Studies*, 10, 35-41.

Podola B, Melkonian M. 2005. Selective real-time herbicide monitoring by an array chip biosensor employing diverse microalgae. *Journal of Applied Phycology*, 17, 261-271.

Sanders HO. 1969. 25. Toxicity of Pesticides to the Crustacean *Gammarus lacustris.* Bureau of Sport Fisheries and Wildlife. United States Department of the Interior Fish and Wildlife Service, Washington, DC.

TenBrook PL, Palumbo AJ, Fojut TL, Tjeerdema RS, Hann P, Karkoski J. 2009. Methodology for derivation of pesticide water quality criteria for the protection of aquatic life in the Sacramento and San Joaquin River Basins. Phase II: methodology development and derivation of chlorpyrifos criteria. Report prepared for the Central Valley Regional Water Quality Control Board, Rancho Cordova, CA.

TenBrook PL, Tjeerdema RS. 2006. Methodology for derivation of pesticide water quality criteria for the protection of aquatic life in the Sacramento and San Joaquin River Basins. Phase I: Review of existing methodologies. Final Report. Central Valley Regional Water Quality Control Board, Rancho Cordova, CA.

USEPA. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses, PB-85-227049. United States Environmental Protection Agency, National Technical Information Service, Springfield, VA.
USEPA. 1996. Algal Toxicity, Tiers I and II, Ecological Effects Test Guidelines, OPPTS 850.5400, EPA 712/C/96/164. United States Environmental Protection Agency, Washington, DC.
USEPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 4th ed. Office of Water, US Environmental Protection Agency, Washington DC. Section 14.10.2.3.1 of Method 1003.0, the green alga, *Selenastrum capricornutum*, growth test, EPA 821-R-02-013.
USEPA. 2003. Water quality guidance for the Great Lakes system. *Federal Register*, 40.